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Biogas digester liquid—a nutrient supplement for mushroom cultivation

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ABSTRACT

This research attempts to find a new use for lignin and N-rich biogas digester liquid (BDL) as a nutrient supplement during cultivation of pre-packed mushroom bags. Spraying 100 ml BDL/bag/day was found to increase yields of *Pleurotus florida* by 66–100%. This is a new application for the emerging biomass-fed biogas plants and this makes such plants economic and sustainable. A mass balance analysis of nutrients (C,N&P) indicate that the addition of BDL hastened various mushroom growth stages such as formation of pin-heads, fruiting body and first flush by 2–4 days each. A 20% increase in N supply from BDL has found to increase the mushroom yields by 40%. BDL was found to have 100times lower heavy metal content when compared to digested cow slurry and therefore very low accumulation rates. We report for the first time a new use for BDL which allows valorization hitherto considered not possible.

1. Introduction

Soft biomass is being extensively used today and promoted as an alternative feedstock for biogas energy production and greater sustainability. Such biomass feedstocks contain cellulose, hemicellulose, lignin and cell wall proteins (Chanakya and Sreesha, 2012a,b). During anaerobic digestion of these biomass feedstocks (fed as a slurry or powder), most of the cell wall proteins, hemicellulose and a part of the cellulose is converted to biogas. The undigested residue, generally in a slurry form, consists of cellulose and lignin where lignin is considered recalcitrant under anaerobic conditions. This slurry of undigested biomass residue left behind after anaerobic digestion is mostly used as a rich manure or compost. Most often biomass feedstocks do not mix with water and stratify into a liquid and biomass particulate layers and therefore unlike cattle dung biogas plants this results in two outputs a digested particulate phase and a clear digester liquid phase for which there has not been any significant use (Chankya et al., 2009). Similarly in plug-flow reactor (PFR) type biogas plants used for biomass feed stocks, the digested output consists of (1) biogas digester liquid (BDL) and (2) ligno-cellulose rich biogas digester residue, (BDR, Chanakya and Sreesha, 2012a,b). Biogas digester residue can be converted to various value added products such as mushroom (with increase in productivity ~300%; Ganguli and Chanakya, 1994), vermi-compost (Suthar 2010), fiber for fabric (Chanakya and Sreesha, 2012a,b), etc. Thus the bioresource technology and sustainability challenge today lies in finding appropriate and long lasting applications and techniques for use of digester liquid that is currently discarded, utilized poorly and which pose environmental concerns. A similar problem faces those biogas plants fed with municipal solid wastes (MSW) where in after, filtration of digested MSW the filtrate has little use and is generally discarded.

A major fraction of the leachable-N of the overall digested feed mass (BDR + BDL) is found in BDL (>75%, Chanakya and Sreesha, 2012a,b). Similarly, BDL was reported to hold >85% of total-P of in input biomass feedstock (Chanakya and Sreesha, 2012a,b, 2013) making BDL a rich source of nutrients with potential that would ideally have to be recycled or converted to value added products (VAP) in addition to biogas as the main output from a biogas plant (Yamamoto et al., 2006). In spite of its high nutrient value BDL has not been considered as a good nutrient supplement when applied directly. When anaerobic digester liquid is applied directly to soil as fertilizer it can often lead to significant nutrient losses arising from leaching and/or ammonia volatilization on the one side in addition to P immobilization as insoluble phosphates thereby decreasing nutrient recycling efficiency and sometimes threatening overall sustainability (Möller et al., 2008). Therefore, it may be ideal to capture these nutrients, ammonical-N and labile P into another biological system that allows their recycle at a latter stage.

Mushroom cultivation is a million dollar industry in many countries including China, India and SE Asia where raw material and

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preparation of selective compost constitutes major cost input (Van Roestel, 1988) and nutrition in feedstock limits the productivity (Raghunathan and Swaminathan, 2003). Therefore increasing the mushroom productivity and lowering the production cost would increase value addition to mushroom growers (Royse, 2010). Thus use of BDL in mushroom cultivation becomes important considering that adding 10–50% digested feedstock has been shown to treble oyster mushroom yield (Ganguly and Chanakya, 1994) and addition of BDL could also have a similar response. While the potential of using biogas digested residue to enhance *Pleurotes* mushroom yield has been proven and its large 'basidiomycete utilizable' lignin and nutrient has been implicated, BDL that contains similar constituents has not been proven for its mushroom production potential or has not been reported. This study for the first time reports that biogas digester liquid (BDL) can be used to enhance *Pleurotes* mushroom yields thereby increasing resource use efficiency and economic sustainability of biogas plants in Asia. This research has evolved firstly the process requirements to enhance yields of *Pleurotus Florida* grown of paddy straw supplemented with BDL. The effect of various nutrients such as complex-carbon, N and P on the growth and yields were studied for *Pleurotus florida* as well as heavy metal uptake from BDL

2. Materials and methods

2.1. Feedstock and mushroom source

Mass produced ready-to-fruit 1 kg perforated plastic mushroom bags (*Pleurotus florida*) were purchased from Indian Institute of Horticulture Research (IIHR) Bangalore. These pre-packed, ready to fruit bags had well formed mycelial network and provided a fair degree of uniformity and were at a ready-to-fruit stage (30d after inoculation). Each of these bags were placed in plastic trays which in turn were placed in a specially constructed humid chamber covered with jute cloth which maximized humidity control (Gangulli and Chanakya, 1994). Three such bags tagged as control were sprayed daily with 100 ml water and the other 3 bags were sprayed daily with 100 ml of BDL and 10 such trials were carried out to optimize the process.

2.2. Biogas digester liquid

BDL (10L) was taken from the outlet of a functioning plug flow reactor (ASTRA-PFR) fed with leafy biomass (1KgTS/m^3, combination of *Tectona Grandis*, *Syzygium cumini* and *Ficus aurea* leaves). The BDL was filtered through a sieve (50 μm mesh size) and autoclaved and saved for further experimentation. From this stock a 100 ml sample was sprayed daily onto the bags. This spraying served as nutrient medium and also maintained adequate moisture content/humidity.

2.3. Sampling of substrate

Of the 100 ml BDL sprayed daily on the ready-to-fruit bags, around 80–85 ml leached down into trays placed below (totaling 2.5 l in 30d). This was collected everyday stored at −4 °C and termed as 'leachate'. This leachate was tested for various physicochemical analyses like total carbon, TKN, free ammonia and TP

A composite sample of 50 g (paddy straw enclosed in *P. florida* mycelia) was collected from different bags at various intervals till the third flush. These samples were dried in a hot air oven at 90 ± 2 °C. The dried material was later powdered and samples were stored in paper bags for further analysis. These samples were used to determine various parameters such as TKN, TP, total carbon etc. in the ready to fruit mushroom bags in order to estimate residual nutrient status in substrates and consequent uptake efficiencies [mass balance].

2.4. Mushroom growth and harvest

Within a week to 10d of spraying BDL on the bags the pin head initiation occurred along with the first flush that followed immediately after. When the fruiting bodies developed they were harvested as first, second and third flush at intervals of a week. The harvested mushrooms were washed with tap water and dried in a hot air oven at 90 ± 2 °C. The dried mushroom samples were powdered and stored in containers for further analysis (heavy metals, total carbon, nitrogen, total phosphorus, sulphur and protein)

2.5. Physico-chemical analysis

2.5.1. Solid sample analysis

The TOC and nitrogen for solid samples (paddy straw and *P. florida*) was determined using a CHNSO elemental analyzer (Thermo Scientific Flash 2000 Organic Elemental Analyzer) with a thermal conductivity detector with carrier gas and reference gas as helium. For analyses of CHNS, column PQS SS 2 M 6 × 5 mm with an oven temperature of 75 °C was used. Substrate (paddy straw with mycelia) sampled were oven dried, powdered and sieved by a 500 μ mesh and used for CHNS analysis. Total phosphorus in solid samples was estimated as outlined in APHA (1978, phospho-molybdate method). Fat, fiber and ash content of the mushroom were estimated using the procedure outlined in Sadasivam and Manickam (1996). Sequential extraction of mushroom samples (*P. florida*) was adopted to estimate constituents such as water and oxalate soluble fraction, hemicellulose and cellulose was carried out by the method of Chesson (1978).

2.5.2. Liquid sample analysis

In liquid samples (mushroom leachate) TOC was estimated using micro-dichromate oxidation procedure outlined by Maciolek (1962). Total nitrogen and free ammonia were determined using micro-Kjeldahl process and total phosphorus in the mushroom leachate was estimated by vanado-molybdate method (APHA, 1978).

2.5.3. Analysis of heavy metals

Heavy metal taken up by *Pleurotus florida* was estimated by determining the heavy metals contained in digested samples of mushrooms using ICP-OES. Dried mushroom samples were digested in nitric acid (Curdová et al., 2004) and analysis was carried out in Thermo-iCAP 6000 series ICP-OES.

All the analyses were carried out in triplicates and statistical analyses were carried out using R Studio [version 2.15.2 (2012-10-26)] and other worksheet packages.

3. Results and discussions

3.1. Biogas digester liquid

The PFR type biogas plants operated at CST-IISc takes up biomass feedstocks without need for pulverization of feedstock and such digesters produce three outputs namely, biogas, digester liquid and digested residue. The digested residue is rich in lignin (generally recalcitrant under anaerobic conditions) and has C, N locked in it (Chanakya and Sreesha, 2012a,b). The digester liquid however has lower solids content (2.1%, Table 1) and most of the solids is collected as digester residue (BDR) in the process. In the digester liquid most of the carbon is found either in the soluble form as VFA, soluble organics including lignin and tiny particulates wherein the carbon content was found to be 0.8% (Table 1). Around 18.75% of